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Increased Milk Levels of Transforming Growth Factor-α, β1, and β2 During *Escherichia coli*-Induced Mastitis

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ABSTRACT

Among the gram-negative bacteria that cause mastitis, *Escherichia coli* are the most prevalent. The innate immune system provides initial protection against *E. coli* infection by detecting the presence of the foreign pathogens and by mounting an inflammatory response, the latter of which is mediated by cytokines such as IL-1β, IL-8, and tumor necrosis factor (TNF)-α. Although changes in these cytokines during mastitis have been well-described, it is believed that other mediators moderate mammary gland inflammatory responses as well. The growth factors/cytokines transforming growth factor (TGF)-α, TGF-β1, and TGF-β2 are all expressed in the mammary gland and have been implicated in regulating mammary gland development. In other tissues, these growth factors/cytokines have been shown to moderate inflammation. The objective of the current study was to determine whether TGF-α, TGF-β1, and TGF-β2 milk concentrations were altered during the course of *E. coli*-induced mastitis. The contralateral quarters of 11 midlactating Holstein cows were challenged with either saline or 72 cfu of *E. coli*, and milk samples were collected. Basal milk levels of TGF-α, TGF-β1, and TGF-β2 were 98.81 ± 22.69 pg/mL, 3.35 ± 0.49 ng/mL, and 22.36 ± 3.78 ng/mL, respectively. Analysis of whey samples derived from *E. coli*-infected quarters revealed an increase in milk levels of TGF-α within 16 h of challenge, and these increases persisted for an additional 56 h. Elevated TGF-β1 and TGF-β2 milk concentrations were detected in *E. coli*-infected quarters 32 h after challenge, and these elevations were sustained throughout the study. Because TGF-α, TGF-β1, and TGF-β2 have been implicated in mediating inflammatory processes, their induction during mastitis is consistent with a role for these molecules in mediating mammary gland host innate immune responses to infection.

(**Key words:** cytokine, *Escherichia coli*, mastitis, transforming growth factor)

Abbreviation key: EGF = epidermal growth factor, **PGE_2** = prostaglandin E_2 **TGF** = transforming growth factor, **TNF** = tumor necrosis factor.

INTRODUCTION

Gram-negative bacteria are responsible for approximately one-third of all clinical cases of bovine mastitis, and nearly 25% of these cases result in culling or death of the animal (Eberhart, 1984). Of the gram-negative bacteria that cause bovine mastitis, *Escherichia coli* remain the most prevalent (Wilson et al., 1997). The innate immune system represents the first line of active defense in the host response to infection by *E. coli* and other bacterial pathogens and is often characterized by the establishment of a pro-inflammatory state (Bannerman et al., 2004). Cytokines, such as IL-1β, IL-8, and tumor necrosis factor (**TNF**)-α, contribute to the establishment of inflammation by altering vascular permeability, promoting leukocyte recruitment to the site of infection, and inducing hepatic synthesis of acute phase proteins that facilitate complement activation and host detection of bacterial wall products (Dinarello, 1997; Feghali and Wright, 1997). Although the role of these cytokines is well-characterized, these are not the only ones implicated in moderating the inflammatory response. Other cytokines, including IL-10, are of significant importance for their ability to downregulate the pro-inflammatory response (Spits and de Waal Malefyt, 1992), which can become deleterious to the host if it persists and/or becomes excessive (Dinarello, 1997).

Transforming growth factor (**TGF**)-β is a cytokine well known for its effect on cell growth and differentiation. There are 3 known mammalian isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3 (McCartney-Francis et al., 1998). In bovine milk, only TGF-β1 and TGF-β2 have been reported to be detected (Jin et al., 1991; Ginjala and Pakkanen, 1998). Depending on the stage of mammary gland development, TGF-β regulates ductal growth and patterning and alveolar development and functional differentiation (Daniel et al., 2001). This

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influence of TGF-β on the gland is mediated largely by its inhibitory effect on epithelial cell growth and its stimulatory effect on fibroblasts and other stromal cells. In addition to its role in mammary gland development, TGF-β has been shown to moderate inflammation. Although some pro-inflammatory properties have been ascribed to TGF-β, its major role is as a suppressor of immune and inflammatory responses (Letterio and Roberts, 1998; Ashcroft, 1999). Whether TGF-β functions as an activator or suppressor of inflammation is dependent on the location and activation state of the cells that TGF-β is stimulating, as well as the environmental milieu of other cytokines/immunoregulatory factors that are present. The anti-inflammatory properties of TGF-β are characterized by its ability 1) to inhibit macrophage production of chemokines, pro-inflammatory cytokines, nitric oxide, and reactive oxygen intermediates; 2) to limit IFN-γ production; 3) to increase expression of the IL-1 receptor antagonist; and 4) to enhance macrophage clearance of bacterial debris, inflammatory cells, and injured parenchymal cells (Letterio and Roberts, 1998; Ashcroft, 1999). Thus, TGF-β plays a key role in physiological processes associated with mammary gland development and in the pathological processes associated with inflammation and host immune responses.

Transforming growth factor-α, a growth factor/cytokine that is distinct from TGF-β, belongs to the epidermal growth factor (**EGF**) family (Derynck, 1992). This growth factor/cytokine has been implicated as a mediator of wound healing, epithelial proliferation, angiogenesis, and mammary gland morphogenesis. Cells expressing TGF-α include epithelial cells and fibroblasts as well as those of immunological importance, including neutrophils, macrophages, and eosinophils (Calafat et al., 1997). Consistent with its expression in these cells, data indicate a potential role for TGF-α in mediating host innate immune responses, including the ability to directly stimulate IL-8 (Subauste and Proud, 2001) and prostaglandin E_2 (**PGE_2**) production (Bry, 1993) and to induce expression of antimicrobial peptides (Sorensen et al., 2003). Thus, a potential role for TGF-α could be envisioned in the setting of mastitis, whereby this cytokine mediates both the initial inflammatory response and the tissue repair that ensues.

Although much is known about the expression of the classic pro-inflammatory cytokines IL-1β, IL-8, and TNF-α during mastitis (Riollet et al., 2000; Bannerman et al., 2004), little is known about changes in the expression of other cytokines, including TGF-α, TGF-β1, and TGF-β2 during this disease. Because the latter are all known to have pleiotropic properties that reportedly moderate inflammatory responses and the ensuing resolution of inflammation, the expression patterns of TGF-α, TGF-β1, and TGF-β2 were characterized in cattle during the course of *E. coli*-induced mastitis.

MATERIALS AND METHODS

Cows

Eleven healthy, midlactating Holstein cows (257 ± 24.6 DIM) were selected on the basis of milk SCC of <250,000 cells/mL and the absence of detectable bacterial growth from aseptically collected milk samples plated on blood agar plates. The use and care of all animals in this study was approved by the Beltsville Agricultural Research Center's Animal Care and Use Committee.

Intramammary Challenge with *E. coli*

Prior to intramammary challenge, 10 mL of brain heart infusion broth (Becton-Dickinson Diagnostic Systems, Inc., Sparks, MD) was inoculated with *E. coli* strain P4 and incubated for 6 h at 37°C. This strain was originally isolated from a clinical case of mastitis and has been used as a model organism in other studies of mastitis (Bannerman et al., 2004). One milliliter of the incubated culture was transferred to an aerating flask containing 99 mL of tryptic soy broth and incubated overnight at 37°C. After incubation, the flask was placed in an ice water bath and mixed by swirling. A 1-mL aliquot was serially diluted in PBS, and 1 mL of the resulting dilutions was mixed with 9 mL of premelted trypticase soy agar in petri dishes. The plates were allowed to solidify at room temperature and then transferred to a 37°C incubator overnight. The aerating flask containing the stock culture was maintained at 4°C overnight. After determining the concentration of the stock culture based on the prepared pour plates, the stock culture was diluted in PBS to yield a final approximate concentration of 35 cfu/mL.

On the morning of challenge, a milk sample was collected (time 0), and the cows were milked. Immediately after milking, one quarter of each cow was infused with 2 mL of the prepared *E. coli* inoculum, and the corresponding contralateral quarter was infused with 2 mL of PBS. Pour-plating of the final prepared inoculum confirmed that cows received 72 cfu per quarter of *E. coli*. Beginning 8 h after the collection of the time 0 sample, aseptic milk samples were collected from all quarters, serially diluted, and plated on blood agar plates. Following a 16-h incubation at 37°C, the number of cfu was determined. Colonies displaying characteristics similar to those of *E. coli* and that were confirmed to be gram-negative, oxidase-negative rods were initially counted as *E. coli*. Confirmatory biochemical tests were performed by the Maryland Department of Agriculture

Animal Health Section (College Park, MD) using the API 20E Gram-negative identification system (bioMerieux, Inc., Durham, NC) for definitive species identification.

Determination of Milk SCC and Whey Preparation

To quantitate somatic cells, milk samples were heated to 60°C and subsequently maintained at 40°C until counted on an automated cell counter (Fossomatic model 90; Foss Food Technology, Hillerød, Denmark). For the preparation of whey, milk samples were centrifuged at 44,000 $\times g$ at 4°C for 30 min, and the fat layer was removed with a spatula. The skimmed milk was decanted into a clean tube and centrifuged again for 30 min as described previously; the translucent supernatant was collected and stored at –70°C.

ELISA

The levels of BSA, IL-8, and TNF-α in milk were determined by ELISA as previously described (Bannerman et al., 2004). Milk levels of TGF-β1 and TGF-β2 were quantified using commercially available kits (R&D Systems, Inc., Minneapolis, MN) that have been previously validated for use with bovine milk samples (Ginjala and Pakkanen, 1998; Pakkanen, 1998). Samples assayed for TGF-β1 were first activated by incubating undiluted whey with an equal volume of an aqueous solution containing 2.5 *N* acetic acid and 10 *M* urea for 10 min. The reaction was then neutralized by the addition of one-half volume of an aqueous solution containing 2.7 *N* sodium hydroxide and 1 *M* HEPES, and the final reactants were diluted 4-fold with the supplied diluent. Samples assayed for TGF-β2 were first diluted (1:13) in deionized water and subsequently activated according to the manufacturer's instructions. For the determination of milk TGF-α concentrations, undiluted whey samples were directly analyzed using a commercially available ELISA (R&D Systems, Inc.).

Statistical Methods

Repeated measures ANOVA was performed using PROC MIXED (SAS 8.2; SAS Institute, Cary, NC). The experimental treatment (i.e., saline or *E. coli* infusion) factor was specified in the MODEL statement. Measurements taken over time on the same cow were modeled using the REPEATED statement with the TYPE option to specify several [e.g., AR(1), CS] within-cow correlation structures. The most appropriate among the candidate correlation structures was indicated by the minimum value of the AICC fit statistic. Estimate statements were written to compare the mean responses in *E. coli*- or saline-infused quarters at each observed time to the respective pre-infused (time 0) response. For statistical analysis of milk SCC, data were transformed to $\log_{10}$ values. A P value of <0.05 was considered significant.

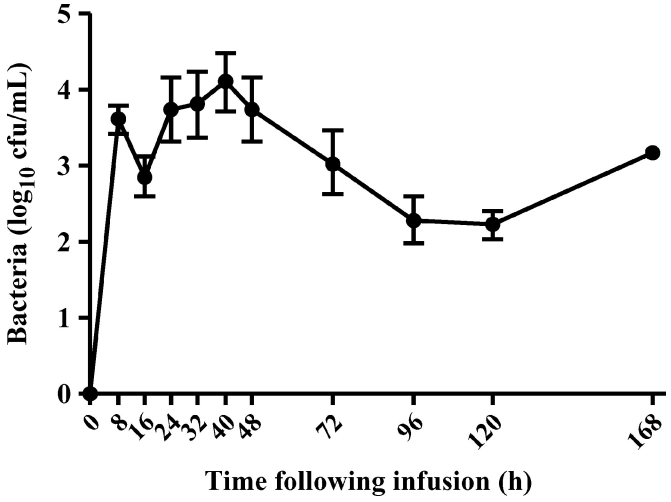

Figure 1. Intramammary bacterial growth following infusion of *Escherichia coli*. Following intramammary infusion of either saline or 72 cfu of *E. coli*, sterile milk samples were collected from all infused quarters at various time points and plated. The mean (±SE) of $\log_{10}$ cfu/mL is shown for those quarters in which viable bacteria were recovered.

RESULTS

Bacterial Growth in *E. coli*-Infected Quarters

Following intramammary infusion of either saline or 72 cfu of *E. coli*, aseptic milk samples were collected throughout the study. The number of cfu of *E. coli* recovered from challenged glands between 8 and 48 h remained relatively steady and reached a peak of 4.10 ± 0.38 $\log_{10}$ cfu/mL at 40 h postinfusion (Figure 1). Saline-infused quarters remained free of detectable pathogens throughout the study.

Intramammary Infection with *E. coli* Elicits both a Systemic and Localized Inflammatory Response

Elevated rectal temperatures were initially detected 8 h after challenge and reached a peak (40.59 ± 0.34°C) 8 h later after which they returned to baseline (time 0) levels (Figure 2). Increased milk SCC in *E. coli*-infected quarters were evident within 16 h of challenge, and milk SCC in these quarters remained elevated throughout the study (Figure 3). Maximum numbers of somatic cells ($46.79 \times 10^6 \pm 6.65 \times 10^6$ cells/mL) were detected in milk 32 h after infection. There was no detectable

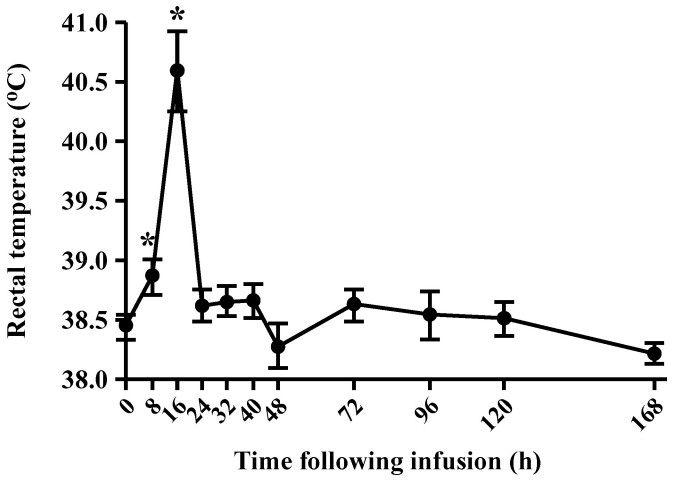

Figure 2. Effect of *Escherichia coli* intramammary infection on core body temperature. As an indicator of a systemic response, rectal temperatures were measured immediately prior to and for various time points following intramammary infection. Mean (±SE) temperature is reported in °C. *$P < 0.05$ (significantly increased compared with time 0).

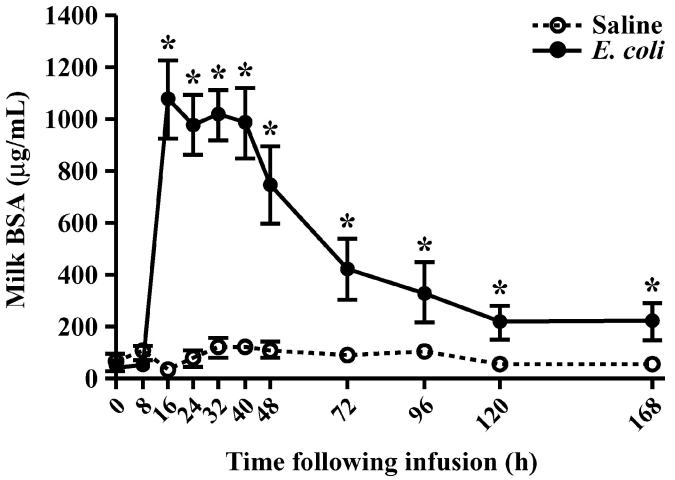

Figure 4. Effect of *Escherichia coli* intramammary infection on milk levels of BSA. As an indicator of changes in the integrity of the blood-mammary gland barrier, BSA concentrations were determined by ELISA in milk samples obtained from quarters immediately prior to and for varying time points following intramammary infusion with saline or *E. coli*. Mean (±SE) BSA levels are reported in μg/mL. *$P < 0.05$ (significantly increased in *E. coli*-infected quarters relative to time 0).

increase in milk SCC in saline-infused quarters throughout the study. In addition to increased SCC, milk obtained from quarters infused with *E. coli* had elevated levels of BSA (Figure 4), an indicator of the breakdown of the vascular endothelial-epithelial barrier in the gland. Increased milk levels of BSA were detected within 16 h of challenge and persisted throughout the study. In contrast, there was no detectable change in BSA concentrations in milk obtained from quarters infused with saline.

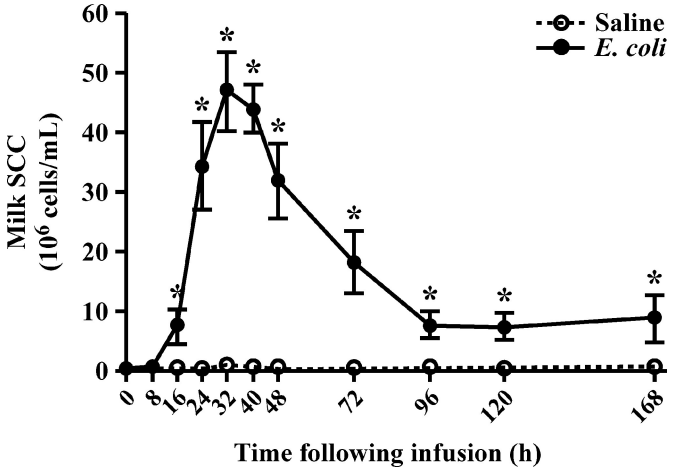

Figure 3. Effect of intramammary infection with *Escherichia coli* on milk SCC. Milk SCC were quantified in milk samples collected from both infected and saline-infused quarters throughout the study. Mean (±SE) milk SCC are reported in millions/mL. *$P < 0.05$ (significantly increased in *E. coli*-infected quarters relative to time 0).

Intramammary *E. coli* Infection Induces the Production of IL-8 and TNF-α

Intramammary infection with *E. coli* induced an increase in milk concentrations of IL-8 and TNF-α within 16 h of challenge, and elevated levels of milk IL-8 and TNF-α persisted through 24 and 40 h, respectively (Figure 5). Maximal elevations in milk concentrations of IL-8 (395.9 ± 82.9 pg/mL) and TNF-α (16.86 ± 3.94 ng/mL) were observed 16 h after bacterial infusion. There were no quantifiable changes in the milk levels of either cytokine in saline-infused quarters.

Increased Levels of TGF-α in Milk During Intramammary *E. coli* Infection

Prior to challenge (time 0), detectable levels of TGF-α were present in the milk of all quarters (Figure 6). There were no significant differences ($P = 0.8437$) in the basal (time 0) milk levels of TGF-α in quarters subsequently infused with saline (103.96 ± 36.99 pg/mL) vs. those infused with *E. coli* (94.60 ± 29.66 pg/mL). Within 16 h of challenge, an increase in milk TGF-α levels above basal (time 0) levels were evident in quarters infected with *E. coli*. The increase in milk TGF-α in *E. coli*-infected quarters peaked 40 h after challenge and reached a mean maximum concentration of 500.53 ± 98.41 pg/mL. Elevated levels of TGF-α in these quarters persisted until 72 h post-challenge.

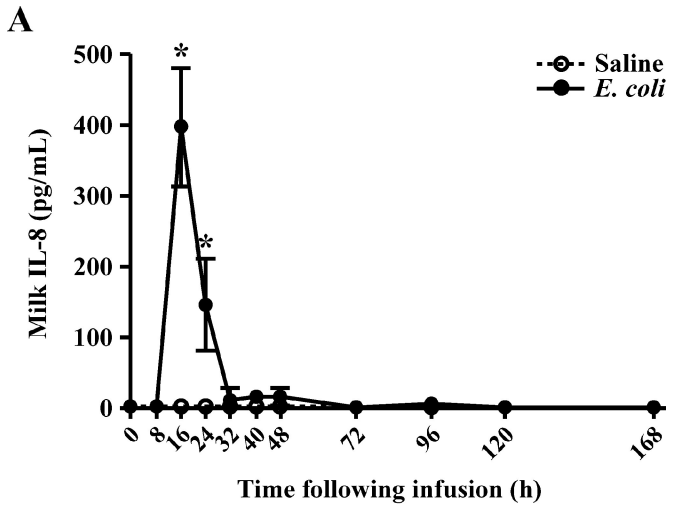

Figure 5. Effect of intramammary *Escherichia coli* infection on milk concentrations of IL-8 and tumor necrosis factor (TNF)-α. ELISA were used to measure the concentrations of IL-8 (A) and TNF-α (B) in milk obtained from quarters infused with saline or *E. coli*. Mean (±SE) IL-8 and TNF-α levels are reported in pg/mL and ng/mL, respectively. *$P < 0.05$ (significantly increased in *E. coli*-infected quarters relative to time 0).

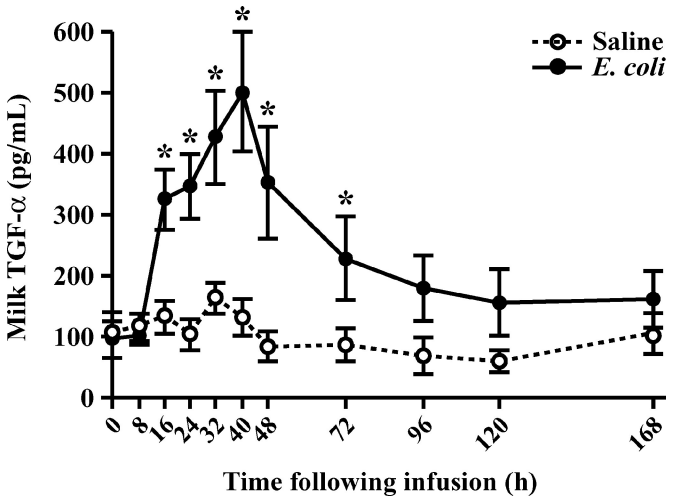

Figure 6. Effect of intramammary infection with *Escherichia coli* on milk concentrations of transforming growth factor (TGF)-α. Levels of TGF-α in milk following intramammary bacterial infusion or saline infusion were determined by ELISA. Mean (±SE) TGF-α concentrations are reported in pg/mL. *$P < 0.05$ (significantly increased in *E. coli*-infected quarters relative to time 0).

Intramammary *E. coli* Infection Elicits Sustained Elevations in Milk Concentrations of TGF-β1 and TGF-β2

Similar to TGF-α, detectable levels of TGF-β1 and TGF-β2 were present in milk under basal conditions prior to challenge (time 0) (Figure 7). There were no significant differences ($P = 0.4829$) in milk concentrations of TGF-β1 in quarters infused with saline (3.71 ± 0.76 ng/mL) or *E. coli* (2.99 ± 0.64 ng/mL) prior to challenge (time 0). Similarly, there were no significant differences ($P = 0.9926$) in initial (time 0) milk TGF-β2 concentrations in quarters subsequently challenged with either saline or *E. coli* (22.33 ± 5.43 ng/mL vs. 22.40 ± 5.54 ng/mL, respectively). Relative to time 0, increased TGF-β1 and TGF-β2 levels were detected in milk from *E. coli*-infected quarters within 32 h, and the levels of both cytokines remained elevated throughout the study. Maximal concentrations of milk TGF-β1 (13.27 ± 1.94 ng/mL) and TGF-β2 (72.99 ± 16.09 ng/mL) were detected in milk 48 and 96 h postinfection, respectively.

With the exception of the 48-h time point, milk TGF-β1 levels in saline infused quarters remained unchanged from the initial basal (time 0) concentrations (Figure 7A). At 48 h, a slight but significant ($P = 0.0433$) transient increase in milk TGF-β1 was detected in saline control quarters; however, this level was significantly ($P = 0.0056$) less than that detected in *E. coli*-infused quarters at the same time point. Similarly, transient increases in milk TGF-β2 concentrations were detected in quarters infused with saline at 32 ($P = 0.0021$) and 40 h ($P = 0.0142$) (Figure 7B).

DISCUSSION

Intramammary infection with *E. coli* elicited a systemic response characterized by the induction of fever and a localized response characterized by pro-inflammatory cytokine production, breakdown of the integrity of the mammary vascular endothelial-epithelial barrier, and elevated milk SCC. In *E. coli*-challenged quarters, maximal increases in the pro-inflammatory cytokine TNF-α, a potent inducer of fever, were observed at

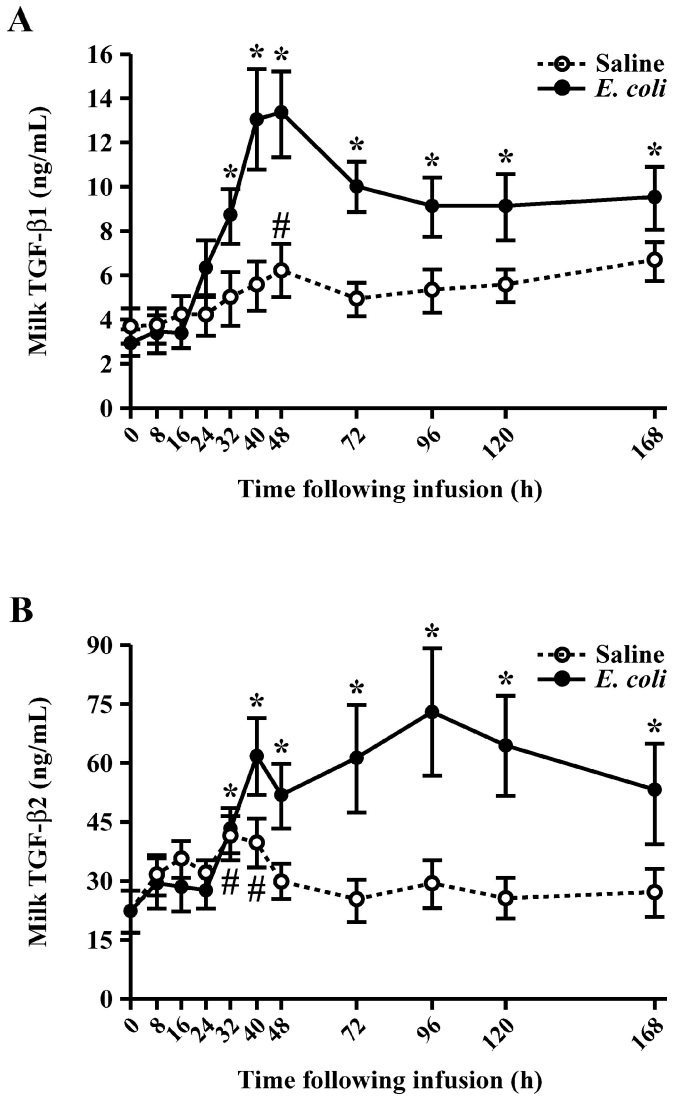

Figure 7. Effect of *Escherichia coli* intramammary infection on the levels of transforming growth factor (TGF)-β1 and TGF-β2 in milk. The concentrations of TGF-β1 (A) and TGF-β2 (B) in milk obtained from quarters infused with either saline or *E. coli* were determined by ELISA. Mean (±SE) concentrations are reported in ng/mL. Significant increases in *E. coli*-infected (*) or saline-infused quarters (#), respectively, relative to time 0 ($P < 0.05$).

16 h postinfection (Figure 5), a time that was temporally coincident with maximal increases in body temperature (Figure 2). Increases in milk SCC were observed within 16 h of *E. coli* infection (Figure 3) and were temporally coincident with increases in milk IL-8 and TNF-α (Figure 5), both of which are chemoattractants (Smart and Casale, 1994; Hammond et al., 1995). The induction of a systemic and localized response reported here, as well as the temporal changes in IL-8 and TNF-α expression, are consistent with previous studies of *E. coli*-induced mastitis (Riollet et al., 2000; Bannerman et al., 2004).

During mastitis, changes in the expression of IL-8 and TNF-α, as well as other cytokines such as IL-1β, have been well characterized (Riollet et al., 2000; Bannerman et al., 2004). The source of these cytokines is mainly from resident cells within the gland, including epithelial cells and macrophages. These cell types have been reported to produce an array of other cytokines that mediate inflammation, including TGF-α (Madtes et al., 1988; Alison et al., 1993) and TGF-β (Maier et al., 1991; Wahl et al., 1991). Further, neutrophils, which are recruited to the gland during mastitis and constitute the majority of the somatic cells in the gland during the early stages of mastitis (Saad and Ostensson, 1990), have also been reported to produce both of these cytokines (Calafat et al., 1997; Chu et al., 2000). In the bovine mammary gland, TGF-α and TGF-β mRNA transcript expression has been detected in the parenchyma (Maier et al., 1991; Sheffield, 1997). In bovine milk, of the 3 known mammalian TGF-β isoforms, only TGF-β1 and TGF-β2 are expressed at measurable levels (Ginjala and Pakkanen, 1998; Pakkanen, 1998). The concentrations of TGF-β1 (3.35 ± 0.49 ng/mL) and TGF-β2 (22.36 ± 3.78 ng/mL) reported here under basal conditions (time 0) (Figure 7) are comparable with those reported by others in bovine milk (Ginjala and Pakkanen, 1998; Pakkanen, 1998). Further, the relative ratio of the amounts of TGF-β1 to TGF-β2 present in bovine milk, with TGF-β2 being the predominant form expressed, is also consistent with a previous study (Jin et al., 1991). Although there are no previous reports on the concentration of TGF-α in bovine milk, the amounts reported here in healthy quarters prior to challenge (98.81 ± 22.69 pg/mL) (Figure 6) are comparable with those in human milk (McPherson and Wagner, 2001).

Transforming growth factor-α has been shown to promote inflammation by upregulating the production of prostaglandins and synergistically enhancing the effects of IL-1β and TNF-α (Bry, 1993; Subauste and Proud, 2001). Similar to the other pro-inflammatory cytokines assayed (i.e., IL-8 and TNF-α) (Figure 5), TGF-α levels in milk increased within 16 h of *E. coli* infection (Figure 6). In contrast to these other cytokines, TGF-α levels were sustained for a longer period and did not return to basal levels until >72 h after challenge. This finding of increased TGF-α protein levels in milk during mastitis is consistent with that of Sheffield (1997), who demonstrated an increase in mammary tissue TGF-α mRNA expression during mastitis. Because the induction of mRNA expression of certain pro-inflammatory cytokines during mastitis can occur in the absence of detectable increases in the corresponding protein, it cannot be assumed that an increase in mRNA expression is necessarily indicative of an increase in

protein. For example, *Staphylococcus aureus*-induced mastitis has been shown to induce TNF-α mRNA expression in milk cells (Riollet et al., 2001; Alluwaimi et al., 2003) without increasing milk TNF-α protein levels (Riollet et al., 2000; Bannerman et al., 2004). The current report directly establishes that TGF-α protein concentrations increase in milk during mastitis.

Although the findings reported here demonstrate an increase in TGF-α during mastitis, the actual effect that this increase has on the gland remains unknown. One may postulate that the increase in TGF-α promotes a pro-inflammatory state and, thus, is a component of the host innate defenses. Initial increases in TGF-α paralleled that of TNF-α, a pro-inflammatory cytokine whose effects are synergistically enhanced by TGF-α (Bry, 1993; Subauste and Proud, 2001). In addition to its role in inflammation, TGF-α promotes tissue repair, mammary epithelial proliferation, and mammary gland morphogenesis (Derynck, 1992). The inflammation that accompanies mastitis can result in injury to the epithelial lining of the gland (Capuco et al., 1986). Thus, TGF-α-mediated epithelial proliferation and tissue remodeling may contribute to gland repair and a return to homeostasis following intramammary infection.

Because a prolonged inflammatory response can result in tissue damage, agents that contribute to a rapid resolution of the inflammatory response are essential for limiting injury to the host. One such agent implicated in limiting the scope of inflammation is TGF-β. The various isoforms of TGF-β, including those expressed in milk, moderate immune effector cell function by inhibiting pro-inflammatory cytokine production, decreasing cell proliferation, and inducing a hyporesponsive state (Ayoub and Yang, 1997; McCartney-Francis et al., 1998). In one study, recombinant human TGF-β1 inhibited bovine blood and milk mononuclear cell activation and IL-2 production (Ayoub and Yang, 1997). Consistent with a counteractive, anti-inflammatory role for TGF-β, increases in the pro-inflammatory cytokines IL-8 and TNF-α (Figure 5) were followed by increases in milk TGF-β1 and TGF-β2 (Figure 7). Whereas IL-8 and TNF-α levels returned to pre-challenge levels within 32 and 48 h of infection, TGF-β1 and TGF-β2 levels remained elevated for >168 h. Although in vitro studies have demonstrated that TGF-β inhibits IL-8 and TNF-α expression (Bogdan et al., 1992; Chen and Manning, 1996), whether TGF-β exerts the same effect in vivo in the setting of mastitis remains unknown.

The present report establishes that intramammary infection with *E. coli* elicits increased TGF-β expression. This is consistent with other reports of elevated TGF-β expression in rats and baboons following systemic infection with *E. coli* (Junger et al., 1995; Ahmad et al., 1997). The contribution of TGF-β to the pathophysiological processes that are operative in the gland during mastitis remains to be defined. Although TGF-β may play a role in dampening the inflammatory response, it has been reported to have pro-inflammatory effects on peripheral blood monocytes that can lead to their activation (Letterio and Roberts, 1998; Ashcroft, 1999). Once monocytes leave the vasculature and take up residence as tissue macrophages, TGF-β effects on these cells become generally suppressive. Again, this supports a potential anti-inflammatory role for TGF-β in the mammary gland. In addition, TGF-β induces extracellular matrix deposition and fibrosis, which contribute to the formation of scar tissue (Shah et al., 1994). Because scarring often develops following mastitis and can lead to permanently reduced milk production, TGF-β may have a role in this process as well.

To our knowledge, the present report is the first to examine the effects of mastitis on milk TGF-β1 and TGF-β2 levels. Further, this is the first study to measure changes in milk TGF-α at the protein level during mastitis. Based on the present findings, we conclude that TGF-α, TGF-β1, and TGF-β2 levels in milk are all increased during the course of mastitis induced by *E. coli*. Given the pleiotropic nature of these cytokines/growth factors in regulating both mammary gland development and inflammation, it is likely that these molecules play a role in innate immune responses to intramammary infection and the ensuing tissue repair and return to homeostasis.

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